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Multiple Forms of Monoamine Oxidase: Functional Significance

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THE enzyme which was later to be called monoamine oxidase (MAO) (monoamine: O_2 oxidoreductase (deaminating), EC 1.4.3.4) was given the name, tyramine oxidase, when first identified by Hare (50) in 1928. A number of parallel observations of the 1930's (11, 68, 91, 92) gradually coalesced into an understanding that "tyramine oxidase," "adrenaline oxidase" and "aliphatic amine oxidase" denoted different properties of one enzyme carrying out the following reaction:

$$R—CH_2—NH_2 + H_2O + \tfrac{1}{2}O_2 \rightarrow R—CHO + H_2O_2 + NH_3$$

On the basis of what was then known about its substrate and inhibitor specificities, it was termed MAO by Zeller (143) to distinguish it from diamine oxidase. Its substrates not only include such "biogenic amines" (44) as the catecholamines and indolealkylamines, to mention but a few, but also many other primary and secondary amines not occurring naturally in the animal organism (9, 10, 27, 39, 143).

Intensive study of the properties of MAO has been made difficult by the fact that it is an insoluble component of the outer membrane of the mitochondrion (101). Nevertheless, enzyme from a number of species has recently been "solubilized" and purified (30, 48, 54, 56, 57, 81, 83, 88, 116, 140) so that in consequence much new information has now been obtained. It is now generally accepted that the enzyme contains 1 mole of flavin cofactor per 120,000 g protein (30, 35, 115, 142), identified as flavin adenine dinucleotide (FAD) (84, 88, 115, 141, 142). Whether the presence of a metal contributes to its activity is not clear (39, 107). The original suggestion that the enzyme might be a copper-containing protein (83) has not received much support, for purified preparations of MAO contain very little of the metal (1, 88, 115, 140) and copper deficiency does not affect enzyme activity (103, 140). However, it now appears that rat liver MAO may depend on a nutritional requirement of iron for its activity (112, 113). This metal has recently been detected in purified enzyme from both rat and pig liver (88, 140). The properties of solubilized, purified MAO have been reviewed by Tipton (118).

Indirect Evidence for Multiplicity

MAO is widely distributed among both vertebrates and invertebrates (10). Over the years a large body of indirect evidence has gradually accumulated to

suggest that, depending on the source, there may be more than one form of the enzyme. Alles and Heegard (2) studied liver enzyme from several species with a large number of substrates and noted substantial differences in their relative rates of oxidation. The work of Werle and Roewer (122), who were able to identify enzyme from different animal sources capable of oxidizing either aliphatic or aromatic monoamines, helped to support the impression that more than one enzyme variant might exist. A suggestion that MAO represents an interrelated group of enzymes, each member possessing slightly different substrate specificities, was made by Satake (100) who further indicated that the precise constitution of the group might vary from tissue to tissue. Since these early observations, much additional indirect evidence that MAO exists in multiple forms has accrued during the course of observations on the effects of inhibitors on enzyme activity; they vary according to the substrate employed (39, 40–42, 49, 110, 139). Thermal stability characteristics (139) and pH optima (5, 139) of MAO preparations also vary with the substrate employed and have similarly been used as criteria for multiplicity (39, 42). Before 1967, when Youdim and Sandler (136) demonstrated the first clear-cut separation of multiple forms of MAO, only Gorkin (38) had been able to achieve partial chromatographic separation of two forms of a rat liver mitochondrial enzyme capable of deaminating *p*-nitrophenylethylamine and *m*-nitro-*p*-hydroxybenzylamine respectively.

Separation and Isolation of Multiple Forms of Monoamine Oxidase (MAO)

It is now 5 years since Youdim and Sandler first used polyacrylamide gel electrophoresis to separate the different constituents of solubilized MAO preparations from rat liver and human placenta. At about the same time, Kim and D'Iorio (65), separated different forms of the enzyme from rat liver and brain, with cellulose acetate electrophoresis. Since these observations, multiple forms of MAO have repeatedly been identified by electrophoresis in a variety of different tissues (table 1).

Apart from this type of physicochemical resolution, Ragland (93) reported the partial separation on Sephadex G-200 of enzyme fractions from rat, beef and rabbit liver that possessed varying activity towards different substrates. This series of observations conflicts in part, however, with later authors who showed that the isoenzymes of rat liver (129) and beef adrenal gland (120) MAO, when separated electrophoretically, do not vary significantly in their molecular weights. The multiple forms of the beef liver enzyme do appear to differ in their molecular weights, however, forming a polymeric series (35).

Molecular Weight Studies

The molecular weight of either native MAO or its constituent isoenzymes has been measured by a number of investigators. However, as shown in table 2, there is little agreement among them. Estimates vary from 1.2×10^5 to 1.25×10^6. Rat liver MAO variants (129) and beef brain isoenzymes (48) appear to have similar molecular weights, 3×10^5 and 4×10^5 respectively. In contrast, the

TABLE 1

The electrophoretic pattern of multiple forms of solubilized mitochondrial MAO from a variety of different species and tissues

Tissue	"Anodic"	"Cathodic"	References
Rat liver	2, 3, 4, 7	1	1, 24, 65, 104, 132
brain	4, 3	1	104, 131
uterus	1	2	108
heart	4, 3	0	104, 138
Human brain	3, 2	1	22, 82
liver	4	1	24
endometrium	2	1	108
placenta	2	1	136
platelets	1	0	21
Pig brain	1	0	119
liver	3		88
Beef brain	2		48
liver	1	2	35
adrenal medulla	4	1	120
Monkey intestine	1		81
Chick brain	5, 6	1	103, 104
Xenopus larvae	3, 2		4

The figures indicate the number of bands of activity claimed by various authors to migrate towards the anode or the cathode.

multiple enzyme forms of beef (35) and pig (88) liver, and rat uterus (23) form a polymeric series. These discrepancies are not fully understood and may relate to methods used to prepare enzyme from the mitochondrial outer membrane. Agreement is only obtained if the molecular weight is calculated per mole of flavin. It seems that 1 mole of flavin, only recently characterized as 8α-(cysteinyl) FAD (64a, 121a), is associated with 1.2–1.5 $\times$ 10^5 g MAO protein (30, 35, 88, 115, 142). Obviously further information such as amino acid content, partial specific volume and diffusion constant is necessary to establish the true molecular weight of MAO.

Properties of Multiple Forms of Monoamine Oxidase (MAO)

Although there is now a considerable body of evidence attesting to the physicochemical separation of solubilized mitochondrial MAO into its constituent multiple forms, their direct comparison from species to species and tissue to tissue is only possible to a limited extent. The overall pattern is extremely complex and to what extent observed differences hinge on variations in solubilization procedure, which tend to be rather drastic, has yet to emerge; but whichever procedure is employed, evidence of heterogeneity is obtained, whether by electrophoresis (table 1), ultracentrifugation (35), gel filtration (93) or investigation of antigenicity (52).

As will be seen presently, these isoenzymes, if it is permitted to call them such, show marked but consistent variation in activity toward a variety of different

TABLE 2

Molecular weights of multiple forms

Tissue	"Anodic"					"Cathodic"		References
	Native enzyme	1	2	3	4	1	2	
Rat liver	1.5×10^5 or 3×10^5	3×10^5	3.1×10^5	2.9×10^5	2.9×10^5			107, 129, 140
brain	2.3×10^5							131
uterus		1.75×10^5				3.7×10^5	6.8×10^5	23, 108
Human platelets	2.6×10^5	2.6×10^5						21
brain	4×10^5							82
Bovine brain	4×10^5	4×10^5	4×10^5					48, 82
liver	2.8×10^5					4.24×10^5	1.25×10^6	35
adrenal	3×10^5							120
Pig brain	1.2×10^5 or 4.2×10^5	1.2×10^5						116, 119
liver	1.1×10^5 or 2.9×10^5							88

substrates and, in particular, toward a number of biologically active monoamines widely regarded as neurotransmitters (13).

Substrate Specificities of the Multiple Forms

The substrate specificities of the multiple forms of MAO so far identified (table 1) show wide variation (*e.g.*, 125, 132); the pattern of a particular tissue may be similar in different species as in brain and liver from human or rat (24, 125, 132). Bands which have been arbitrarily designated MAO_1 and MAO_2 are most active towards tyramine and kynuramine whereas benzylamine, dopamine and tryptamine are less actively metabolized (24, 125). Beef adrenal medulla isoenzymes (117, 120) appear to have substrate specificities almost identical with those from human and rat liver. This is rather surprising since considerable species and organ differences have been reported for MAO preparations from other sources (see ref. 118 for review). Harada *et al.* (48) have reported that while the two beef brain MAO components they identified show a similar rate of kynuramine oxidation, enzyme 1 has a higher activity towards tyramine and tryptamine, and enzyme 2 a higher activity towards normetanephrine and benzylamine. Rat liver MAO was separated electrophoretically into two components by one group of workers (65, 105) who noted that the first acts on benzylamine and the second on both benzylamine and 5-hydroxytryptamine. The same group (28) has confirmed these results and also shown that kynuramine oxidation is carried out predominantly by the "benzylamine only" component. Rat brain MAO appears to be more active towards benzylamine than to tyramine (131).

Tetrazolium salts have been used by Shih and Eiduson (103, 104) to identify and to measure the activity of the multiple forms of MAO of chick brain, separated electrophoretically. In the embryonic or newborn chick, their number was greater, with several different substrates, than in the mature animal and varied with the substrate employed: with tyramine and kynuramine 5 were observed but none was seen with tryptamine. A rather similar phenomenon was noted during studies on enzyme from the tadpole of the South African clawed toad, (*Xenopus laevis*) with 5-hydroxytryptamine as substrate (4), which was interpreted as reflecting differences in substrate specificity. However, recent studies (72, 135) have demonstrated that the concentrations of tetrazolium salts used in the assay, which was originally devised for the histochemical localization of MAO (34), strongly inhibit MAO activity as measured by radioassay techniques. Moreover, sites of enzyme activity detected on polyacrylamide gel by the tetrazolium technique do not necessarily coincide in all cases with bands of activity measured by such radioassay (135). Nor does the intensity of tetrazolium reduction reflect the radiochemical activity of the different forms. Thus the use of tetrazolium salts for measuring the activity of multiple forms of MAO during electrophoresis of solubilized preparations or, presumably, in histological studies, can lead to an erroneous assessment of activity, to a degree depending on the substrate employed.

Three components have been separated from solubilized beef liver MAO (35). That termed fraction A, which is unstable, migrates to the anode; components 1

and 2 are more stable and migrate to cathode. The beef isoenzymes are thus somewhat different from those of human and rat liver (22, 125, 131, 132), which migrate predominantly towards the anode. Component 2 is considerably more active towards tryptamine and tyramine than either component 1 or fraction A. Fraction A oxidizes norepinephrine and epinephrine at a slower rate than component 1 and 2. The antigenic properties of all three forms have recently been studied (51–53, 55) and found to be identical. About 80% of solubilized beef brain mitochondrial MAO is antigenically similar to liver MAO (52, 55). The other 20%, which does not cross-react with the antibody to liver MAO, has been separated and shown to differ also in respect to inhibition characteristics. No details of the substrate specificity of this enzyme, tentatively named brain "specific" MAO, have so far been given.

Other workers have shown that human (22) and rat (131) brain MAO separate into four bands of activity on polyacrylamide gel electrophoresis, three "anodic" bands and a single "cathodic" band, termed MAO_B (125, 132) which has also been identified in other sites (*e.g.*, 108). An interesting and consistent observation in all the tissues studied (120, 125) is the unique substrate specificity of this band which appears to be highly specific for the oxidation of dopamine. For this and other reasons (see below) it has provisionally been termed "dopamine" monoamine oxidase" (125). The highest activity of this enzyme variant so far observed is in the basal ganglia of the human brain (22). At the present time there is insufficient evidence to identify it as the brain "specific" MAO reported by Hartman and his colleagues (52, 59) even though there appear to be certain similarities, *e.g.*, resistance to heat and MAO inhibitors. Further observations are urgently required.

Rat uterine multiple forms possess certain properties (23) in common with the beef liver enzyme (35), including molecular weight, electrophoretic mobility and existence in polymeric forms. However their substrate specificities differ.

Recently, solubilized partially purified MAO from rat heart mitochondria, has also been subjected to polyacrylamide gel electrophoresis. Preliminary results (138) point to the presence of three bands of activity, one at the origin and two migrating towards the anode. It has not so far been possible to demonstrate a "cathodic" band, unlike rat or human brain and liver (22, 125). Substrate specificities of these bands are under investigation at the present time.

Multiple Forms: Fact or Fiction?

It has been suggested that the multiple forms of solubilized MAO might represent a single enzyme having varying amounts of membrane material bound to it (121). It is difficult to believe, however, that the diverse physicochemical procedures employed for solubilization produce the same membrane fragmentation pattern. Ultrasonic oscillation (121) of mitochondria results in membrane fragments varying from 50 to 200 Å in diameter. They may be separated partially by density gradient electrophoresis (1) or completely by polyacrylamide gel electrophoresis into fractions which differ in substrate specificity (see above). Solubilization of the enzyme with detergents (30, 127) also yields particles 70 Å in diameter

which, on electrophoresis, yield multiple bands of activity. In fact, the isoenzyme pattern of MAO from rat tissue or chick brain appears to be constant whether sonication or non-ionic detergent is used for solubilization (65, 103, 127). What is intriguing is that if all these findings are artifacts of the solubilization procedure, they are highly consistent and reproducible (and indeed, worthy of study in their own right). Ultrasonic fragmentation of the outer mitochondrial membrane might have been expected to result in an infinite gradation of particles rather than, say, the constant five bands of rat and human liver (125), or, even more remarkable in the context of the present discussion, the single band of activity of the human platelet (27).

The molecular basis of the multiple forms of MAO awaits unequivocal explanation (126). Tipton *et al.* (120) recently presented data to indicate that the isoenzymes of beef adrenal MAO might derive from the binding of varying amounts of phospholipid phosphate to the enzyme protein. This concept has latterly been extended to the rat liver enzyme (117, 130); although the phospholipid content was somewhat lower for all five forms (130), the earlier studies on beef adrenal enzyme were, in general, confirmed. However, there seems to be little relationship between phospholipid phosphate content and electrophoretic mobility; the lowest amounts of this material were both in the fastest moving "anodic" band (MAO_4) and in the "cathodic" band MAO_R ("dopamine monoamine oxidase"), while the highest concentrations were associated with band MAO_1 which remains at the origin during electrophoresis. Thus the presence of multiple forms cannot be ascribed solely to the presence of varying amounts of phospholipid phosphate.

The studies of Tipton *et al.* (120), indicate that the lipid moiety may not only stabilize the enzyme but is necessary for its activity. Treatment by prolonged dialysis against 1.25% Triton X-100 resulted not only in partial inactivation of the enzyme but also in the disappearance of multiple forms on electrophoresis and their replacement by a single band. Similar findings have been obtained with 1% sodium dodecyl sulphate (129). Treatment with phospholipase again resulted in a loss of activity and the appearance of a single band on electrophoresis. On the other hand, it has been reported that treatment of a purified preparation of beef kidney MAO with phospholipase A does not affect enzyme activity (30).

Purified pig liver MAO appears to have an extremely high affinity for the phospholipid, cardiolipin (37, 89). If the multiple forms of MAO stem from the binding of different amounts of lipid to enzyme protein, the question arises whether the same phenomenon might occur *in vivo*. The modification of the *in vivo* properties of enzymes by association with lipid is, of course, not an unknown phenomenon (33, 102). Such a property could, in the present case, be associated with the existence of different types of mitochondria (12, 86) or the presence of cell-specific MAO. Thus, it is possible to explain the different substrate specificities of intraneuronal and extraneuronal MAO (3, 59, 60) on the basis of a single enzyme existing in different lipid environments (117). Further investigations of lipid content should obviously be carried out on MAO from human platelet, (21) pig brain (119) and monkey intestine (81) which appear to be homogeneous on polyacrylamide gel electrophoresis.

Differences in polymeric state of a single active subunit as the basis for the multiplicity of beef liver and rat uterine MAO have been described (35, 88, 108). Such a phenomenon may, however, be excluded as an explanation for the multiple forms of rat liver or beef adrenal MAO since solubilized preparations of both behave in a homogeneous manner on gel filtration and ultracentrifugation (120, 129, 140) having a molecular weight of approximately 300,000. The suggestion (36) that electrophoretic multiplicity might arise *via* the oxidation of sulfhydryl groups (30, 36, 115) can perhaps be discounted, since the presence of 0.1 M 2-mercaptoethanol does not change the electrophoretic pattern (129). An association of dissimilar subunits provides another possible explanation. Rat liver enzyme can be dissociated into subunits of similar molecular weight (78,000) with either 8 M urea or 1 % sodium dodecyl sulphate (129). These subunits do not differ, however, in their electrophoretic mobility on polyacrylamide gel (129), although it is always possible that the electrophoretic method used to attempt to separate them was unsuitable. This situation is analogous to that of the mitochondrial malate dehydrogenase isoenzymes which were originally thought to arise from conformational changes in a protein molecule consisting of similar subunits (29, 63); but the enzyme has recently been shown to possess more than one type of subunit (75). Much work remains to be done, including the preparation of homogeneous isoenzyme for analysis of amino acid content and other parameters, before a definitive solution is obtained to this problem.

Perhaps the most important of the unanswered questions is to what extent the findings *in vitro* are mirrored *in vivo*. The assessment *in vivo* of MAO activity presents difficult problems which have not entirely been solved. A number of approaches are on record of which a recent method (112) with ^{14}C-*n*-pentylamine is a promising addition. Some evidence for the existence *in vivo* of MAO isoenzymes in the intact mitochondrion comes from studies of human brain and rat liver after inhibitor pretreatment (25, 98, 133, 134) where subsequently harvested isoenzymes manifest different degrees of inhibition; had enzyme multiplicity been a result of the preparative procedure, it might have been expected that all bands of activity would be inhibited to a similar extent. Similar results have been reported for rat brain enzyme (37, 85) where the existence *in vivo* of two forms, termed A and B, has been inferred. Enzyme A, which was sensitive to clorgyline (M & B 9302) (61), oxidatively deaminated tyramine and 5-hydroxytryptamine with facility; enzyme B, on the other hand, which was relatively insensitive to clorgyline, oxidized tyramine but not 5-hydroxytryptamine. The proportions of these two isoenzymes in different anatomical locations have also been shown to vary. Superior cervical ganglion was characterized by the presence of about 90 % type A and 10 % type B. In contrast, rat pineal gland contained 15 % type A and 85 % type B. The presence of two enzyme systems has also been inferred from inhibitor studies by other authors (32, 45, 46, 61, 66, 111).

Hormonal Control of Monoamine Oxidase (MAO)

There is increasing evidence to suggest that endocrine gland secretions can influence the activity of MAO (16, 20, 58, 62, 67, 123, 145). The observation of

Southgate *et al.* (109) that MAO activity in the human endometrium increased about 10-fold during the second half of the menstrual cycle appears to be related to progesterone secretion, for the highest activity coincided with peak concentrations of circulating plasma progesterone. Because earlier observations in the experimental animal were inconclusive (16, 62, 67, 123, 145) Holzbauer and Youdim (58) were prompted to reinvestigate the activity of this enzyme during the estrus cycle in various tissues of rat. Brain (amygdala, hippocampus, caudate, nucleus, septum and hypothalamus), adrenal glands and ovaries showed maximal activity during the late proestrus phase with a rapid fall during late estrus and a rise once more during late diestrus. Uterine MAO showed two peaks of activity, one during late proestrus and one during early metestrus. Steroids have been shown to have a significant effect on uterine MAO activity, with estradiol producing a fall and progesterone an increase (16, 20, 43, 123). An attempt was made to compare steroid secretion rates from rat ovaries during the estrus cycle with tissue MAO activity (the hypothalamus was used) (58). There was a short burst of progesterone secretion during early proestrus but the highest secretion rate was observed at met- and early diestrus. However, peak MAO activity did not coincide with it; there appeared to be a latency between ovarian progesterone secretion and enhancement of tissue MAO activity. If estrogens, which are known to have an inhibitory effect *in vitro* (20) bring about a reduction *in vivo* in MAO activity when secreted during the estrus cycle, it is again characterized by a time lag between secretion and action on the enzyme. Yoshinaga *et al.* (124) found the highest secretion rates of estrogens from the ovary to occur during proestrus whereas MAO activity was lowest at the time of late estrus (58). At present, there is insufficient evidence to decide whether the increase in MAO activity observed during the estrus cycle derives, perhaps, from facilitation of MAO protein synthesis or from release from inhibition by an agent such as the estrogens. Of the two, the inhibitor theory is more probable, for enzyme protein generation is likely to be slow; when MAO activity is inhibited with the irreversible inhibitor, phenelzine, for instance, new activity is not observed until about 6 to 8 weeks have elapsed (69).

To what extent do these cyclic variations affect the pattern of multiple forms in the uterus? Collins and Southgate (23) have produced evidence to indicate that the changes in specific activity brought about by hormone treatments derive from quantitative alterations in individual isoenzyme bands. Gel electrophoresis of partially purified uterine enzyme is characterized by a single band of activity migrating towards the anode (MAO_I), but, uniquely, two components migrating to the cathode (MAO_{II} and $_{III}$) (table 1). Preparations of solubilized rat uterine MAO obtained from hormone-treated and control rats have shown the same hormone-induced alterations in activity as the crude homogenates (20, 23) but these changes are characterized by differential alterations of individual isoenzymes. Bands MAO_I and MAO_{II} (108) were found to have similar activity in all the enzyme preparations but the activity of MAO_{III} was much reduced in the estradiol-treated group and increased 10-fold in the progesterone-treated group.

The multiple forms of glutamate dehydrogenase exist as a polymeric series, the state of aggregation of which can be influenced by steroids and other small molec-

ular weight compounds (7). The multiple forms of beef liver MAO similarly appear to form a polymeric series (35). On available evidence, it seems not unlikely that rat uterine MAO isoenzymes might similarly constitute a polymeric series (23, 108, 126). Three peaks of activity have in fact been identified by gel filtration of partially purified enzyme on Sephadex G-200; their molecular weights are 175,800, 371,500, and 675,100. Although their identity with the three electrophoretic bands of enzyme activity seems likely, no attempt has so far been made to cross-relate these findings by measuring the molecular weights of the electrophoretically separated bands. The changes in activity brought about by estradiol and progesterone may derive from shifts in the relative proportions of each. An increase in rat uterine MAO activity is also brought about by pretreatment of the animal with dopa (20). A similar finding has been noted in rabbit blood vessel MAO after the prolonged administration of high doses of dopa (114). This phenomenon, which may perhaps be viewed as an adaptive response to the presence of high concentrations of its substrate, dopamine, may be mediated similarly. However, the increase appears to be confined to MAO_{III}, (23, 108) which is highly specific for dopamine as substrate. It now seems, in fact, as though this isoenzyme is identical with MAO_B and, as mentioned above is a separate enzyme, "dopamine monoamine oxidase" (125, 134) (also see later). Whether the increase in dopamine oxidizing ability bears any relationship to the occasional onset of postmenopausal bleeding in parkinsonian patients during L-dopa treatment (96) is still a matter for speculation.

Functional Significance

It has always appeared likely that MAO possesses more than one physiological substrate. Such a view now seems more tenable since the multiple forms of MAO with their characteristic tissue distribution (table 1) and substrate specificities started to be characterized. Although it is now a truism to assert that MAO plays an important physiological role by controlling the concentration of intracellular monoamines at specific sites (69), the statement serves a useful purpose, for we have then to ask, "Which monoamines and at which site?" And the answer is by no means forthcoming. We need to know because of an important but empirical clinical finding, that certain depressive patients are benefitted by MAO inhibitor therapy. Although many drugs in this group act on other enzyme systems (*e.g.*, 18, 19, 64, 90) and also possess other properties of potential interest, such as an ability to block amine reuptake (54a), so many different types of chemical compound are involved (9, 10, 90) that it seems not unreasonable to suppose that the one obvious property they share, MAO inhibition, is of primary importance in eliciting the clinical response. The experimental study of the MAO inhibitors has so far provided no easy answer to the question of how they bring about a lightening of affect. The data obtained are extremely complex. A comparison of the inhibition pattern of the multiple forms of rat liver MAO for instance, both *in vitro* and *in vivo*, shows wide variation depending on the substrate employed (25). With kynuramine (70), for example, studies *in vitro* have shown that MAO_4 in this organ is more resistant to inhibition by iproniazid, pheniprazine, harmaline

and clorgyline than are the other forms of the enzyme. Similarly, when ^{14}C-tyramine is the substrate (94), the same enzyme form is resistant to inhibition by iproniazid, pargyline and harmaline (25). Multiple forms of MAO prepared from rat liver after pretreatment *in vivo* with clorgyline, tranylcypromine or pargyline (25, 98), once again demonstrate clearly that MAO_4 is more resistant to inhibition, with ^{14}C-tyramine as substrate, than are the other forms. At present, then, indications are that differences *in vitro* in inhibitor affinity to some extent reflect variations *in vivo* and are not merely a result of the preparative procedure.

Conversely, some of the multiple forms are more sensitive to certain inhibitors than others, again to some extent depending on the substrate employed. Rat liver MAO_2, for instance, is extremely vulnerable to the inhibitory action of tranycypromine when tyramine is employed as substrate (25). If this observation can be extrapolated to man, it may prove of clinical importance: the variable hypertensive effect of tyramine in patients treated with different MAO inhibitors is well described, with tranylcypromine being potentially one of the most dangerous (8). If an isoenzyme in man similar to rat MAO_2 were responsible for a major proportion of the *in vivo* inactivation of a substrate such as tyramine, its selective inactivation by a relatively specific inhibitor such as tranylcypromine might have deleterious consequences in the presence of an excess of that substrate.

Although depressive illness often responds to treatment with an MAO inhibitor such as tranylcypromine, it does not follow that any abnormality of MAO itself exists. Indeed brain areas obtained at autopsy from depressed geriatric patients show closely similar activity to corresponding samples from non-depressed subjects, with at least four different substrates (22, 133, 134). When patients in this clinical group were treated with three different MAO inhibitors, tranylcypromine, isocarboxazid and clorgyline, they showed striking variations in pattern of brain MAO inhibition depending both on the substrate and inhibitor employed (98, 134). After tranylcypromine pretreatment, the oxidation of ^{14}C-dopamine was inhibited in all brain areas investigated to an extent significantly greater than that of other substances; after isocarboxazid, on the other hand, ^{14}C-dopamine oxidation was least affected. The finding with tranylcypromine is of considerable interest for it has recently been shown (6), that tranylcypromine causes a significantly greater increase in dopamine concentration in human caudate nucleus and hypothalamus than isocarboxazid or clorgyline.

As with the findings in rat liver, it is tempting to explain these data on the basis of differing sensitivity *in vivo* of the MAO isoenzymes to the drugs employed. There is a considerable body of indirect evidence to indicate that clorgyline possesses a differential inhibitory effect on multiple forms of MAO from different tissues (45, 46, 61) including human brain (22). This particular drug provides a firm precedent for a type of compound which possesses potential therapeutic advantages over conventional "blunderbuss" MAO inhibitor treatment while at the same time providing an investigatory tool of great power.

We have expressed the view (22, 98) that the therapeutic effectiveness of MAO inhibitors is likely to rely on the accumulation of a particular amine substrate at a specific site in the brain. To identify this site is our eventual aim; but it already

seems possible to hazard an explanation as to why some MAO inhibiting drugs are therapeutically effective but others are not (25, 134). The answer may well lie in terms of differential inhibition of the MAO isoenzymes. By mapping brain MAO according to its isoenzymes and their substrate and inhibitor specificities, we hope to pinpoint sites with particular substrate preferences and eventually be in a position to synthesize specific inhibitors tailored to an individual isoenzyme band at a particular anatomical site.

The four isoenzymes prepared from human brain possess markedly different substrate and inhibitor specificities (22, 125, 134); and perhaps of equal note, the activity of an individual band tends to vary remarkably from area to area (22). Thus, the activity of MAO_R (22) in the basal ganglia is more than 70 times greater than that of the same band in the cerebral cortex when using dopamine as substrate. MAO_R, the band which migrates to the cathode, is probably a special case, however. Indeed, it seems not unlikely that it is a separate enzyme (125): apart from its peculiar migration characteristics in an electrical field, compared with the other forms of MAO it is more heat stable (22, 131), possesses a lower pH optimum (131, 132), is relatively resistant to all inhibitors so far investigated apart from tranylcypromine (125, 130, 131, 132) and possesses an extremely low K_m for dopamine (126, 130). On these grounds, therefore, it has been given the tentative name of "dopamine monoamine oxidase" (125).

Although tranylcypromine is an effective antidepressant drug (73), other MAO inhibitors inactive against MAO_R such as isocarboxazid (125, 134) or clorgyline (125, 134) have a similar therapeutic action (6). It thus seems unlikely that inhibition of MAO_R plays a central role in the ability of these drugs to lighten affect.

Apart from its role in the central nervous system, another function of MAO is to inactivate potentially toxic dietary monoamines, as foreshadowed by Blaschko (9) many years ago. Within this context, clorgyline itself, despite its differential inhibitory action, retains the most severe side-effect of the MAO inhibitors; it interferes with tyramine degradation (71) so that patients eating cheese or other foodstuffs with a high tyramine content (76) are liable to a sudden and dangerous rise in blood pressure (8, 76). It is, of course, entirely possible that a build-up of central nervous system tyramine (15) is responsible for the therapeutic benefit achieved with MAO inhibitors; but tyramine is only one of a number of possible candidates, *e.g.*, octopamine (14, 79, 80), tryptamine (14, 77), which have recently been demonstrated in the nervous system and, together with the catecholamines and 5-hydroxytryptamine will need to be taken into the reckoning in identifying the biochemical lesion in depression.

The hypertensive response to tyramine-containing food (76) during MAO inhibitor therapy tends to be somewhat variable; while some subjects are extremely sensitive, others seem to ingest tyramine with impunity. Observations have recently been made which may shed some light on this problem. Although it is customary to consider oxidative deamination as the only degradation mechanism for tyramine worthy of serious consideration, there have been reports from two centers (106, 128) focusing attention on a second metabolic pathway for oral tyramine *via* conjugation. When MAO is inhibited, it is likely that this second

pathway possesses the ability to act as a safely valve mechanism. In fact, a group of subjects exists within the population (with tyramine-sensitive migraine (47), although this characteristic is not perhaps germane to the present discussion) in whom this second pathway is defective (106, 128). Preliminary observations (97) indicate that depressive patients who have manifested severe hypertensive reactions while on MAO inhibitor therapy are unable to conjugate oral tyramine to any large extent. If this observation can be confirmed, it may well be advantageous to carry out a "tyramine tolerance test" to investigate conjugating ability before instituting MAO inhibitor therapy.

Apart from the conjugation defect, there are indications that MAO itself may be inhibited in some migrainous patients, to judge from platelet studies on a small number of affected subjects (99). Collins and Sandler (21), who established that human platelet MAO is unique for this species, in possessing but a single molecular form, have pointed out, however, that studies of platelet MAO activity cannot be employed as indices of overall MAO status, despite opinions to the contrary (94, 144): the qualitative and quantitative variations in MAO activity in different anatomical sites and their varied responses to inhibitors make the platelet enzyme representative only of itself. Similarly, even though gut mucosal MAO activity has been employed as an indicator of whole body MAO pattern (74), local disease (17) or, presumably tissue isoenzyme variation, make extrapolation from the particular to the general an unsafe procedure.

The proceedings of a recent 3-day meeting devoted solely to MAO (26) attest to current widespread interest in this enzyme. Some of the outstanding questions have been mentioned in the course of the present review but there are many others which continue to elude us. Why does human platelet MAO (21) possess a very similar substrate specificity spectrum to plasma benzylamine oxidase (78)? Why do MAO inhibitors possess an antianginal action (90) and do antianginal agents such as prenylamine (137) act by virtue of their MAO inhibiting action? Does thyroidal MAO possess the additional function of generating hydrogen peroxide in order to generate free iodine (31)? Does the product inhibition demonstrated by certain aldehydes generated by the action of MAO (95) have any control function? What is the peculiar nature of pineal MAO (134)? But dwarfing these more minor issues is the unsolved problem of to what extent MAO functions as a control mechanism of catecholamine action. We have rather grown used to thinking of the enzyme as a somewhat crude intracellular disposer of waste monoamine, with the reuptake mechanism acting as a primary system of defense against amine excess at the receptor site. With our latter-day knowledge of the multiple forms and their different substrate specificities, the nature of the back-up offered by MAO obviously needs re-evaluation. It may well be that the enzyme provides a greater measure of fine control and discrimination than so far has been appreciated. Obtaining answers to such outstanding questions is likely to occupy us for many years to come.

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